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Research Article

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Estimation of Radioprotective Effects of *Garcinia Indica* Methanol Extract in Swiss Albino Mice

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ABSTRACT

The radio protective effectiveness of methanol extract of *Garcinia indica* against whole body gamma radiation was premeditated in Swiss albino mice. The oral administration of *Garcinia indica* extract at 800 mg / kg body weight / day for 15 consecutive days before whole body exposure to radiation was found to be effective with the LD_{50/30} values of 7.21 and 8.83 Gy for irradiation alone and *Garcinia indica* and irradiation group, respectively, giving a dose reduction factor of 1.42. This effect of *Garcinia indica* as the modulation of the radiation-induced decrease of reduced glutathione and the radiation-induced increase in lipid per oxidation assessed in the liver and the blood.

Keywords: Radioprotection / *Garcinia indica*, CFU-S/LD_{50/30}, GSH, LPO, Gamma Radiation.

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Introduction

A search for the chemical agents that are able to protect human beings from ionizing radiation is a key issue in radiation biology [1]. Radiation produces various International Journal of Chemistry and Pharmaceutical Sciences

pathological changes in living systems and these changes were reduced with the help of certain synthetic chemicals such as cys-teine, [2] cysteamine, [3] lipoic acid [4] and

deoxyspergualin [5]. But clinical applications of these compounds are very few owing to their high toxicity at optimum dose level. Recently, the interest has been developed in search for potential drugs, especially, of herbal origins, which are capable of modifying immune and radiation responses without their side effects. Several studies concerning radioprotection have been conducted on vitamins [6–8]. The fruits of *Garcinia indica* have been suggested in the Indian system of medicine for a number of diseases. These include its usefulness as an infusion, in skin rashes caused by allergies, treatment of burns, to

2. Materials and Methods

Animals: Male Swiss albino mice, 6–8 weeks old with 25 gm body weight from an inbred colony were used for the present study. Animals were maintained under controlled conditions of temperature (27°C) and light (12 hr dark: 12 hr light) in an animal house, and were provided standard mice feed and water. For irradiation, animals were restrained in well-ventilated boxes and exposed whole-body to gamma radiation at the distance of 75.5 cm. from the source to deliver the dose rate of 1.32 Gy/min.

Plant collection and extraction: The fruits were collected separated from matured fruits, shade dried, broken into small pieces and powdered coarsely. The 500 g of dry fruits sample which was later coarsely powdered in a Willy Mill to 60-mesh size and used for solvent extraction in Soxhlet apparatus and successively extracted with methanol by for 24 hrs. The extract was concentrated using rotary vacuum evaporator. The extract was used for total phenol and flavonoids content and also for estimation of antioxidant through various chemical assays [14–15]. For the different concentrations, a known amount of extract was suspended in distilled water, and 0.1 ml of extract suspension was given to each mouse by oral gavage.

Experimental plan: Acute drug toxicity is to determine the acute toxicity of *Garcinia indica* extract, the animals were divided into 4 groups of 10 each and extract was given orally to them at the concentration of 200, 400, 800 and 1200 mg/kg body weight/day for 15 consecutive days. The mice were observed continuously for 30 days to determine the toxicity of extract in the form of mortality or any other sign, if occurs. Determination of optimum dose of *Garcinia indica* extract against radiation, for the selection of optimum dose of extract against radiation, animals were given 200, 400, 800 and 1200 mg/ kg body weight/day extract for 15 consecutive days. Thirty minutes after the last administration, these animals were exposed to 8 Gy gamma radiations. The reduced glutathione (GSH) and lipid peroxidation (LPO) levels in liver and blood were estimated after 30 minutes of radiation exposure. The optimum dose thus obtained was used for further investigation. Reduced glutathione (GSH) assay is the hepatic level of reduced glutathione (GSH) was determined by the method as

relieve sunstroke, remedy for dysentery and mucous diarrhea, an appetizer, liver tonic, to allay thirst and as a cardiotoxic. Garcinol a polyisoprenylated benzophenones, has antioxidative, chelating, free radical scavenging, anti glycation, anticancer, anti inflammatory, and anti ulcer activities [10–13]. Based on the properties and significance of *Garcinia indica*, the present study has been undertaken to investigate the radio protective efficacy of methanol extract of *Garcinia indica* against radiation induced sickness, change in body weight and animal survivability.

described by Moron *et al.* [16] GSH content in blood was measured spectro photometrically using Ellmans reagent (DTNB) as a coloring reagent as per the method described by Beutler *et al.* [17] The absorbance was read at 412 nm using a UV-VIS Spectrophotometer. Lipid peroxidation (LPO) assay: The lipid peroxidation level in liver and serum was measured in terms of Thiobar-bituric Acid Reactive Substances [TBARS] by the method of Ohkawa *et al.* [18] The absorbance was read at 532 nm. Dose reduction factor is the protective capacity of plant extract is expressed as dose reduction factor. It can be calculated by dividing the LD_{50/30} of *Garcinia indica* and irradiation by LD_{50/30} of irradiation alone animals. Irradiation alone Group the animals were exposed to 6, 8 and 10 Gy of Gamma rays and observed for 30 days to record the mortality and signs of radiation sickness. Experimental Group is the Extract and Irradiation in animals of this group were given extract orally at the dose level of 800 mg/kg body weight/day for 15 consecutive days and exposed to 6, 8, and 10 Gy of gamma rays after the last administration. The animals were observed for 30 days and radiation sickness as well as mortality was recorded in similar manner as it was recorded in irradiation alone group.

Modification of radiation response is the animals selected for this study were divided into two groups. Animals of one group (experimental) were administered *Garcinia indica* extract orally (800mg/kg body weight/day for 15 consecutive days), and the irradiation alone group received double distilled water (volume equal to extract). After 30 minutes of last treatment all the animals were exposed to different doses of gamma radiation (6, 8 and 10 Gy). Body weight is the general condition and body weights of the mice in all groups were observed daily. The per cent change in body-weight in each group of mice was recorded every day by dividing the average body weight of those mice on the first day of treatment as described elsewhere [19]. Survival assay is the selection in Mice of both groups (irradiation alone as well as experimental) exposed to 6, 8 and 10 Gy gamma radiation were checked daily for 30 days and the percentage of mice surviving 30 days of exposure against each radiation dose was used to construct survival-dose response curves.

3. Results and Discussion

Treatment with *Garcinia indica* extract for 15 consecutive

days in mice did not produce any toxic effect. Rather, these

animals showed an increase in body weight at 30 days as compared to sham irradiated animals. A significant decrease in GSH content was observed in irradiation alone animals (Irradiation alone) whereas, *Garcinia indica* and irradiation showed a significant increase in GSH content (blood as well as liver) at various concentrations of

Garcinia indica extract. However, maximum increase in GSH content was observed in the animals pre-treated with 800 mg/kg body weight/day extract and irradiated (Table 1). An increase in TBARS level in liver and serum was also evident in irradiation alone animals.

Radioprotective Effects of *Garcinia indica* Extract

Table 1: Reduced glutathione (GSH) and Lipid per oxidation (LPO) levels in blood and liver of Swiss albino mice with & without *Garcinia indica* extract treatment and exposed to radiation (8 Gy). E= Extract

Treatment	GSH		LPO	
	Blood (g/ml)	Liver (mole/gm)	Blood (nmole/ml)	Liver (nmole/mg)
Irradiated normal (std)	1.70	54.80	1.32	2.24
E200mg	1.70	54.92	1.25	2.23
E400mg	1.72	55.26	1.22	2.20
E800mg	1.77	55.94	1.17	2.14
E1200mg	1.74	55.63	1.20	2.19
Radiation 8Gy	0.678	31.22	3.82	6.32
E200mg+8Gy	0.746	33.68	3.64	6.04
E400mg+8Gy	0.870	37.91	3.43	5.82
E800mg+8Gy	0.963	39.05	3.02	5.64
E1200mg+8Gy	0.917	38.89	3.18	5.71

Although, no significant difference was noticed in such levels in irradiated and extract treated animals. But, a significant dose dependent decrease was registered in extract pre-treated irradiated animals. However, the maximum decline in LPO level was measured in the animals pre-treated with 800 mg/kg body weight/day RE (Table 1). Therefore, 800 mg/kg body weight/day extract was used for detailed study. In the present study, it was observed that pre-treatment of extract enhanced the survival of mice exposed to different doses of gamma radiation. The severity of the radiation sickness was dose dependent and 35% of the animals died within 30 days post irradiation with 6 Gy, whereas, 100% mortality was observed on day 12 and 08 in animals of irradiation alone groups after

exposure to 8 and 10 Gy respectively. The survivability in 6 Gy experimental groups was 100% but it decreased to 58 and 27% in experimental groups after irradiation with 8 and 10 Gy respectively (Fig. 1). Regression analysis of survival data showed 7.21 and 8.83 Gy LD_{50/30} values for irradiation alone and *extract* and irradiation producing a DRF as 1.42. Maximum body weight loss was 26% and minimum loss was 12% in irradiation alone groups whereas, in experimental groups it was 20% and 2% in their respective groups. Not only this, but the extract and irradiation showed 16% (6 Gy), 8% (8 Gy) and 11.5% (10 Gy) increase in their body weight than the initial ones at day 30 post irradiation (Fig. 2).

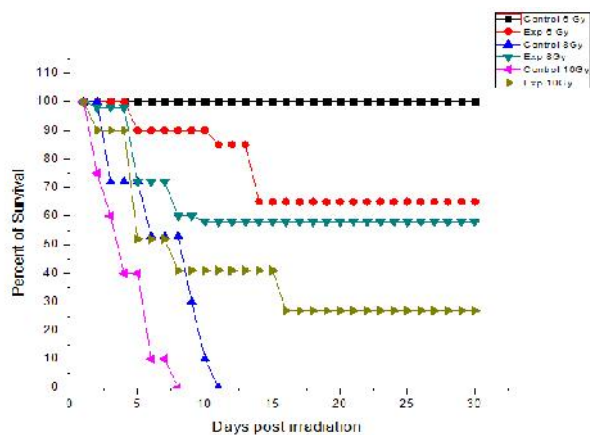


Figure 1: Percent survival of Swiss albino mice after exposure to Gy gamma rays

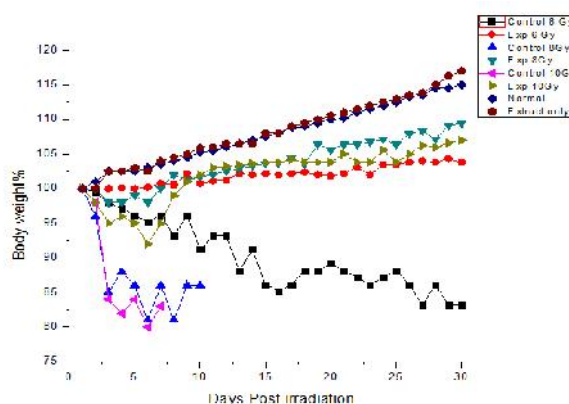


Figure 2: Per cent change in body weight of Swiss albino mice

The results of the present study indicate that pre treatment of *Garcinia indica* extract protect the hematopoietic tissues in mice from the lethal effects of ionizing radiation. The

radio-protective effect of extract was demonstrated by determining the LD_{50/30} values (DRF=1.42). A significant radioprotection was observed when 800 mg/kg body

weight/day extract was given orally for 15 consecutive days before radiation exposure. The free radicals generated during the radiolysis of water play the most important role in the direct biological damage induced by ionizing radiation²⁰. Under normal conditions, the inherent defense system including glutathione and anti-oxidant enzymes protects against the oxidative damage. GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation²¹. GSH is a versatile protector and executes its radio-protective function through free radical scavenging, restoration of the damaged molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state²². A significant decrease in GSH content in liver and blood was observed following gamma irradiation (8 Gy). The oral administration of extract did not influence the endogenous GSH content significantly, but it protects the GSH depletion due to irradiation. These results suggest that endogenous non-protein sulfhydryl content (GSH) is

maintained by the extract in these extract and irradiation group. The oxidative stress due to the radiation induced free radicals can cause a dramatic fall in the hepatic GSH, which overwhelms the cellular defence and lead to membrane lipid per oxidation and a loss of protective thiols²³. GSH might be reacting with the peroxide intermediates; since peroxide intermediates stimulate further lipid per-oxidation by autocatalysis and enhance the damage. Thus the results of the present study suggest that extract pre-treatment provides protection against radiation induced sickness, body weight, mortality and it also maintains GSH and LPO levels in blood and liver. It has been reported that plant flavonoids that show antioxidant activity *in vitro* also function as antioxidants as *in vivo*, and their radio protective effect may be attributed to their radical scavenging activity [24]. The radio protective effect of *Garcinia indica* extract may be assigned to its antioxidant properties as it contains pro vitamin A (-carotene), vitamin C and riboflavin.

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